Somatolactogens, Somatomedins, and Immunity[1]

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ABSTRACT

The neuroendocrine and immune systems participate as active partners in host homeostatic and defense mechanisms. This partnership involves a complex intercommunication system employing an array of shared ligands and receptors. Hormones of the somatolactogen family have marked influences on immune events in vivo, including the maintenance of lymphoid tissue cellularity, the promotion of DNA synthesis in these tissues, and the stimulation of a number of immune effector mechanisms. Both growth hormone and prolactin function to promote erythropoiesis and DNA synthesis in bone marrow precursors. Our results have shown that the somatolactogens and a member of the somatomedin family, IGF-I, are particularly effective in modulating the effector functions in phagocytic cells, including the production of reactive oxygen intermediates and tumor necrosis factor-α and the oxygen-dependent killing of bacteria. Evidence indicating a role of IGF-I in modulating immune functions is more recent but nonetheless compelling. Accumulated data suggest that somatolactogenic hormones, as well as one member of the somatomedins, are produced by cells of the immune system and can regulate local immune events. Although the molecular mechanisms by which the somatolactogens and somatomedins exert their effects on immune tissues are only now being explored, the pleiotropic nature of these effects suggests that these hormones participate at endocrine, paracrine, and perhaps autocrine sites of action.

(**Key words**: growth hormone, prolactin, insulin-like growth factor-I, immunity)

Abbreviation key: **GH** = growth hormone, **IFN-γ** = interferon-γ, **IL** = interleukin, **PL** = placental lactogen, **PMN** = polymorphonuclear leukocyte, **PRL** = prolactin, **TNF-α** = tumor necrosis factor-α.

INTRODUCTION

Food animal producers have the objective of delivering a high quality product at moderate cost to a consumer who is increasingly concerned that this product be aesthetically acceptable. Within the past 50 yr, advances have been considerable in defining the optimal environmental and nutritional requirements in production systems. However, many advances have been offset by an unacceptable level of infectious and noninfectious diseases associated with production. The realization that the immune system is a fully integrated physiological system, subject to external and internal stimuli mediated through neuroendocrine control, provides the conceptual basis for exploiting the immune system of domestic animals for enhanced disease resistance. The use of biological response modifiers and genetically engineered vaccines may soon make this aspiration a reality. Our research interests have focused on endogenous products as modulators of immune function. More recently, we have concentrated on the effects of the somatolacto-

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genic hormone family and one of their endogenous mediators, somatomedin-C (IGF-I) on immune functions. Such studies are particularly timely in view of the potential medical and agricultural uses of recombinant growth hormone (GH). This article reviews some of the information supporting a role for these hormones in maintaining and modulating immune functions. These topics have been periodically reviewed (37, 54, 55, 56).

The Somatolactogen Hormone Family

Prolactin (PRL), GH, and placental lactogen (PL) are now considered to belong to the somatolactogen family of hormones by virtue of a number of shared biological, immunological, and structural features (73, 83). These three hormones have similar size (190 to 199 aa), a similar globular protein structure, and approximately equivalent α-helical content; each possesses two homologous disulfide bonds. All three are now thought to have evolved from a common ancestral gene through a gene duplication event, giving rise to separate GH and PRL lineages. The nature of subsequent duplication events seems to have varied among species; rat and bovine PL genes arise from the ancestral PRL gene, whereas the human PL gene arose from duplication of the primitive GH gene. Despite these shared structural, evolutionary, and functional features, the somatolactogens are synthesized in a disparate, tissue- specific manner; GH and PRL are synthesized in the anterior pituitary, but PL is produced by the placental syncytiotrophoblast. In addition, although members of this family exhibit hormone-specific biological properties, they also share to varying degrees, depending on the species, characteristics of both lactogenic and somatotropic hormones.

Somatolactogens and Immune Tissues

Endocrinology textbooks invariably discuss the classic effects of the somatolactogenic hormone family. For GH, these include the stimulation of hepatic glycogenolysis, the activation of lipolysis in adipose tissue, and the enhancement of amino acid incorporation into muscle protein. Prolactin, at least in mammals, is considered to be primarily a lactogenic hormone, stimulating lactogenesis and mammary growth and development. However, this emphasis on the lactogenic effects of PRL may have obscured other potentially important functions of this hormone, such as the promotion of male reproductive tract and prenatal lung development (8, 9). Placental lactogen seems to have evolved to play roles intermediate to those of GH and PRL during pregnancy, stimulating fetal growth and inducing mammary growth (6). Yet some of the earliest observations on GH and PRL in hypophysectomized animals showed that these hormones were critically involved in the maintenance of lymphoid organ size and cellularity.

Following the original observation in 1930 that hypophysectomy led to involution of the thymus gland, a substantial research effort has been exerted in exploring the role of pituitary hormones in regulating activities of the immune system [reviewed by Berczi and Nagy (5) and Kelley (55, 56)]. Both ACTH, acting via the adrenal cortex, and LH, acting via testosterone, can induce thymic involution; reviewed in Kelley (56)]. The concept of pituitary hormones acting as thymotropic, rather than thymolytic, agents has been explored in a number of model systems. The administration of GH to hypophysectomized rats reversed growth defects and caused particular enhancement of thymic growth and thymic nucleic acid synthesis [reviewed by Berczi and Nagy (5) and Kelley (55)]. However, these early fundamental observations were offset by a host of conflicting observations, leading Dougherty (27) to decide from a review of 195 papers that no definitive conclusions cold be drawn on the effects of hypophysectomy or hormone replacement therapy on immune tissues.

Further development of this concept had to await a better understanding of the immune system and the development of better model systems and purified hormone preparations. The potential limitations of the hypophysectomized model were countered by the demonstration that administration of an antiserum to pituitary extracts, or a GH antiserum, to intact mice to develop thymic atrophy and wasting disease. Also, the pituitary-deficient, Snell-Bagg dwarf mouse had a defect in antibody synthesis that could be reversed by administration of GH [reviewed by Berczi and Nagy (5) and Kelley (55, 56)]. By the 1970s, data from a number of laboratories [reviewed by Berczi

and Nagy (5)] confirmed that spleen, thymus, and lymph node tissue of hypophysectomized animals became atrophic, had reduced nucleic acid synthesis, and were deficient in at least some aspects of the immune response. A major development in the study of somatolactogens in immunobiology came from Berczi's group; they showed that antibody synthesis and contact sensitivity reactions were reduced in hypophysectomized rats and that these effects could be reversed by administration of GH or PRL (80) or PL (6). A comparable demonstration in a domesticated species was made by Roth et al. (93), who found that the thymic atrophy in dwarf Weimeraner dogs could be reversed by GH administration. More recently, the immune defects in pituitary-deficient mice have been shown to extend to the myeloid compartment and to affect a spectrum of myeloid progenitors (76).

Secretion of GH is maximal around puberty and then declines subsequently with aging (55, 56). This age-associated reduction in GH is associated with a loss in cortical thymocytes, thymic involution, and a significant reduction in immune events associated with T cells (47, 55, 106). We (57) have shown that the implantation of a syngeneic pituitary tumor (GH_3 cells) into aged rats, which secrete both GH and PRL, reconstituted histologically normal thymus glands in those aged rats. This restoration was accompanied by augmented T-lymphocyte interleukin (IL)-2 synthesis and increased in vitro proliferative responses of peripheral T lymphocytes to lectins (57). More recently, GH_3 cells, in addition to promoting thymic growth, were found to overcome a key T-cell differentiation block in the thymus of aged rats by promoting the differentiation of double negative cells ($CD4^-$ $CD8^-$) into the double positive phenotype ($CD4^+$ $CD8^+$) (67). A possible mechanism for the effects of the somatolactogens on thymic development is through the ability to stimulate the synthesis of the thymic hormone, thymulin, which influences intra- and extrathymic T-cell differentiation. Growth hormone and PRL stimulate the synthesis of thymulin from thymic epithelial cells (22), and GH injections in vivo augment thymulin secretion in aged dogs (45). Significantly, mice that are transgenic for rat GH contain more thymic epithelial cells (24). Recent evidence demonstrating significant positive effects of GH on engraftment of T-cell progenitors in mice with severe combined immune deficiency (77) suggests that GH may also influence migration of T-cell precursors to the thymus gland.

Somatolactogens and Hemopoiesis

In addition to the decline in cellularity and nucleic acid synthesis of thymus and spleen, hypophysectomy has significant effects on the generation of hemopoietic precursors. This phenomenon has been best defined for erythroid progenitors and was first observed in hypophysectomized rats; these rats, when placed in hypobaric chambers, failed to show the significant rise in hemoglobin or erythrocyte content that was exhibited by control rats and, in addition, did not develop the pronounced bone marrow hyperplasia characteristic of rats undergoing pronounced erythrocyte synthesis [reviewed by Berczi and Nagy (5)]. These effects could be partially reversed by the administration of GH or PRL (79). A more complete characterization of hemopoietic dysfunction in hypophysectomized rats has been undertaken by Berczi and Nagy (4, 5), who found that these rats exhibited normochromic-normocytic anemia, leukopenia, and thrombocytopenia. A general role for an effect of anterior pituitary hormones in the development of hemopoietic precursors is supported by the demonstration of impaired DNA and RNA synthesis in the bone marrow of hypophysectomized rats. Convincing evidence that these effects were due to pituitary insufficiency was provided when hemopoietic parameters and bone marrow nucleic acid synthesis could be normalized by treatment with GH, PRL, or PL (4, 6, 80) and the finding by Jepson and McGarry (52) that GH enhanced erythropoiesis and lymphocytosis in the bone marrow of human hypopituitary dwarfs (5).

Growth hormone also augments the in vitro maturation of erythrocytes derived from bone marrow cells differentiated with erythropoietin (46) and stimulates the proliferation of virus-infected leukemia cells. In view of these results, it is somewhat surprising that long-term bST treatment in dairy cows has been reported to result in a reduced hematocrit (2, 14, 17, 88). Perhaps this discrepancy is due to an increase in plasma volume of cows injected

with bST, as has been reported in rodents (39), although this postulate is not supported by a recent study (35) on the short-term effects of bST in dairy cows. Nonetheless, a role of bST in modulating granulopoiesis in lactating dairy cows is suggested by the finding that bST treatment induced a significant increase in the neutrophil fraction in peripheral blood (14, 17).

Somatolactogens and Lymphocyte Function

Human mononuclear cells have around 7000 membrane-bound, high affinity receptors for GH; the affinity constant was 2×10^9 M^{-1} (60). Growth hormone receptors are also present on thymocytes, transformed lymphocytes, and peripheral blood mononuclear cells (66) although their distribution on mononuclear cell subsets, such as NK (natural killer) cells, B cells, macrophages, and $CD4^+$ or $CD8^+$ T cells, is largely unknown. The expression of PRL receptors on lymphocytes is poorly characterized. The number of PRL receptors on lymphocytes (~360 per cell) (95) is small relative to receptor expression in liver or breast tissue. Recent studies (33, 84) of PRL receptor gene and protein expression in a rat T-cell line stimulated with PRL suggest, however, that receptor concentration is regulated by concentration of the homologous ligand. No studies to date report PL receptor levels on lymphocytes, but, at least in humans, PL can bind to the PRL receptor. Both GH and PRL receptors have now been classified, on the basis of amino acid homology in the ligand-binding domains, as members of a hematopoietin cytokine receptor superfamily, which includes the receptors for IL-2, IL-3, IL-4, IL-6, IL-7, and erythropoietin (20). This homology further strengthens the concept of GH and PRL as functional ligands in the growth, differentiation, and function of lymphoid cells.

Growth hormone consistently augments the in vitro proliferation of transformed (71) and normal (61) lymphoid cells. Results have been disparate when normal lymphocytes have been stimulated to proliferate in the presence of GH (61, 71). These disparities may arise from a number of sources, such as the use of partially purified hormone preparations, the failure to employ an appropriate range of hormone concentrations, or the presence of GH or GH-binding protein in serum preparations (56). With the single exception of the rat Nb2 T-lymphocyte line, the direct in vitro effects of PRL on immune cells are also difficult to characterize (9), probably for many of the reasons mentioned. However, two novel approaches have demonstrated the significance of both these hormones in lymphocyte proliferation events and have also highlighted the importance of lymphocytes as a source of classic neuroendocrine hormones. Weigent et al. (111) used antisense oligonucleotides to GH mRNA to target endogenous de novo synthesis of GH by lymphocytes and found that lectin-induced lymphocyte proliferation was abolished. Similar, Bernton et al. (10) and Sabharwal et al. (96) found that the addition of low concentrations of antisera to pituitary PRL to mitogen-stimulated human lymphocytes abrogated DNA synthesis and cell proliferation. Clevenger et al. (19) used a series of eukaryotic expression vectors to express wild-type PRL, PRL lacking the signal sequence for translocation into the endoplasmic reticulum, or chimeric PRL containing a nuclear translocation sequence in the Nb2 T-cell line. Stimulation with IL-2 resulted in proliferation only in those transfected cells expressing the wild-type or nuclear translocation sequences, demonstrating that both extracellular and intranuclear PRL can synergize with IL-2 in augmenting the proliferation of this cell line. The finding that cells transfected with PRL sequences targeted to the nucleus, but not those expressing the wild-type sequence, could proliferate in the presence of IL-2 and antiserum to PRL suggests a role for PRL within the nucleus.

In contrast to varying effects in vitro, GH and PRL consistently augment a number of immune responses when given in vivo to hypopituitary animals. These enhanced responses include antibody synthesis and skin graft rejection (5, 55, 80, 81), the development of adjuvant arthritis (7), the activity of NK cells (98), and lectin-induced T-cell proliferation and IL-2 synthesis (24, 71). Treatment of dairy cows with recombinant bST for 38 wk enhanced T-cell proliferative responses induced by concanavalin A [Figure 1; (14, 15)] and resulted in higher serum IgG and IgA concentrations (16). The in vitro proliferative response of T cells to a mitogenic lectin is

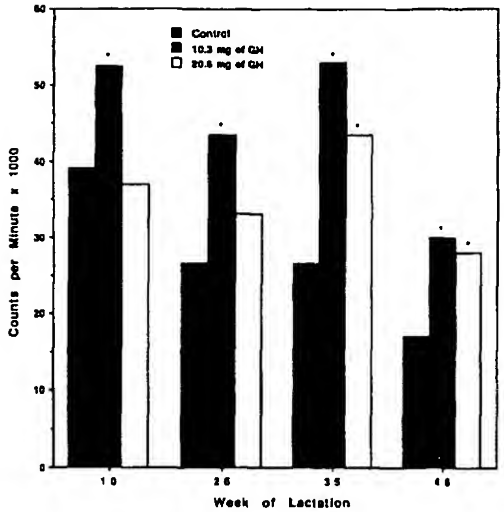

Figure 1. Recombinant bST enhances the proliferative responses of peripheral blood lymphocytes of dairy cows to concanavalin A. Lactating Holsteins were either untreated (control) or treated daily with the indicated concentrations of recombinant bST GH. Data are least squares means, pooled over sample week, and are expressed as counts per minute × 10^{-3}. Data are from Burton (14) and Burton et al. (15).

used as one indicator of cell-mediated immune function. However, these cows did not display changes in dinitrochlorobenzene-induced contact sensitivity responses (14). Treatment of dairy heifers with bST has reduced the inhibition of mitogen-induced proliferation of lymphocytes cultured at higher temperatures (34), although comparable results in heat-stressed lactating dairy cows were not evident (35). Experiments with intact rodents, administered dopamine agonists [e.g., bromocriptine or pergolide, reviewed by Bernton et al. (9)] to suppress endogenous PRL, showed that immunocompetence suppression comparable with that induced following hypophysectomy could be induced. Thus, rats treated with bromocriptine have decreased antibody responses to sheep erythrocytes, decreased contact sensitivity response, and suppressed development of adjuvant arthritis and experimental allergic encephalitis. These effects could be reversed by treatment with PRL or GH (5, 9). An important finding from in vivo studies was that mice treated with bromocriptine had a significantly higher mortality rate following infection with *Listeria monocytogenes*, resulting from an inability to generate activated macrophages because of suppressed production of interferon-γ (IFN-γ) from activated T cells. These changes could be reversed by exogenous PRL (11).

Somatolactogens and Phagocytic Cell Functions

Macrophages are phagocytic cells that are critical for the induction and expansion of a number of immune responses (1, 104). Activated macrophages can be triggered to produce reactive oxygen intermediates that nonspecifically kill ingested bacteria. In addition, activated macrophages process and present bacterial antigens to T cells, express class II antigens of the major histocompatibility complex, kill tumor cells, and secrete a number of monokines, such as IL-1 and tumor necrosis factor-α (TNF-α). Our interest in macrophages arose from an early observation that GH-treated macrophages acquired morphological characteristics of activated macrophages [reviewed by Kelley (55), 56)]. In addition to this change in morphology, in vitro GH treatment of porcine monocyte-derived macrophages primed these cells for enhanced release of the reactive oxygen intermediate, superoxide anion (O_2^-), in response to stimulation with opsonized zymosan (29). As little as 50 ng of GH/ml significantly increased the production of O_2^- and this production could be totally abrogated by preincubation with a specific antibody to GH. Macrophages of hypophysectomized rats could also be primed in vivo by injection of various amounts of either native porcine GH recombinant porcine GH or native rat GH. These findings were extended to another phagocytic cell type, the polymorphonuclear leukocyte (PMN), by demonstrations that GH, PRL, and IGF-I, prime PMN from a variety of species, including the bovine, for enhanced O_2^- secretion [Figure 2; (40, 41)]. These results were particularly significant because they showed that the ability of somatolactogens and somatomedins to enhance production of reactive oxygen intermediates by phagocytic cells was conserved across a number of species.

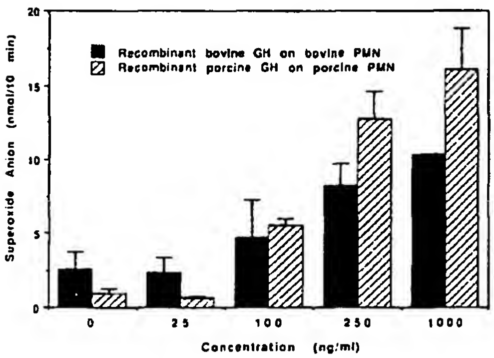

Figure 2. Recombinant bovine growth hormone (GH) or recombinant porcine GH augments O_2^- secretion by polymorphonuclear neutrophil (PMN) of bovine or porcine origin, respectively, in a dose-dependent fashion. Data are from Fu et al. (40).

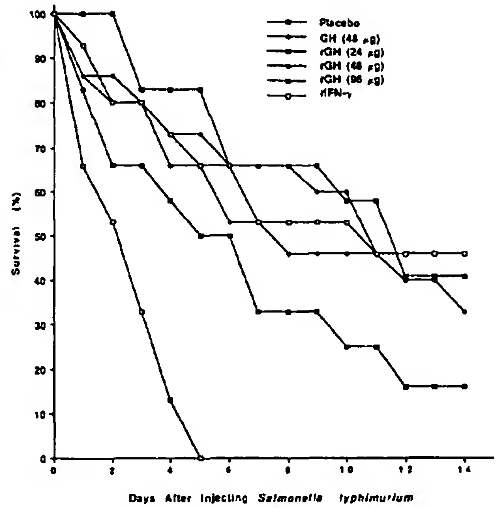

Figure 3. Growth hormone (GH) and interferon-γ (IFN-γ) increase survival of hypophysectomized rats following challenge with *Salmonella typhimurium* on d 0. Rats received the indicated concentrations of native, pituitary-derived porcine GH, recombinant GH (rGH), or recombinant rat IFN-γ for 6 d prior to challenge and for 6 d after infection. Data are from Edwards et al. (32).

Growth hormone treatment in vitro stimulates PMN adhesiveness and O_2^- release in humans (113, 114). Two preliminary reports using dairy cows treated in vivo with recombinant bST (18, 50) showed that similar effects were induced in PMN from milk and peripheral blood. Although those results are preliminary, they indicate that PMN bacterial killing can be enhanced in vivo and that the economic losses caused by postpartum infections, such as *Escherichia coli* mastitis, may be reduced by bST treatment [reviewed by Kelley and Dantzer (58)]. A comparable long-term study in swine showed some effects of GH on PMN migration activity but found no effects on other neutrophil functions, such as chemotaxis, ingestion, antibody-dependent cell-mediated cytotoxicity, or the reduction of cytochrome *c* (65). A growing body of evidence now suggests that both somatotropin and IGF exert chemotactic effects on neutrophils and lymphocytes (65, 103, 114). Species-specific differences occur in somatolactogen-induced priming for O_2^- secretion. Although priming of bovine, porcine, and murine PMN is mediated through the GH receptor, we (40) have shown, by the use of a panel of human GH variants generated by site-directed mutagenesis, that GH priming of human PMN is mediated through the PRL receptor. Consequently, neither bovine nor porcine GH prime human PMN, even at concentrations as high as 10,000 ng/ml.

Three significant in vivo correlates of these in vitro events further bolster evidence for an important immunoregulatory role of GH. First, GH injections enhanced resistance of both intact and hypophysectomized rats following challenge with *Salmonella typhimurium* [Figure 3; (32)]. Although GH had previously been shown to augment several components of both the humoral and cell-mediated immune systems, our study (32) was the first demonstration that GH could actually improve host resistance to an infectious pathogen. Growth hormone was as effective as IFN-γ in enhancing survival and activating peritoneal macrophages to kill *S. typhimurium* in vitro, and these effects were mediated by an enhancement of free radical secretion (28, 30, 32). Second, macrophages from hypophysectomized rats demonstrate an impaired TNF-α response to in vitro lipopolysaccharide stimulation, and this deficit could be partially overcome by in vivo treatment with either GH or IFN-γ (31). Finally, a defect in O_2^- secretion

and TNF-α production in macrophages from aged rats can be reversed by implantation of syngeneic pituitary grafts beneath the kidney capsule (25). These results demonstrate that GH, and probably PRL, regulate an array of physiologically important responses of phagocytic cells.

Somatomedins

In 1957, Salmon and Daughaday proposed that GH acts on skeletal tissues by inducing the formation of a direct acting intermediary growth factor, or somatomedin [reviewed by Sara and Hall (97)]. This hypothesis spawned an active area of research leading to the definition of the somatomedins, or IGF, as mediators anabolic actions of GH in vivo (23). The finding of IGF activity in a wide variety of conditioned media and tissue extracts led to the suggestion that the IGF are not simply endocrine hormones but also function as autocrine or paracrine hormones (94, 97). Purification and amino acid sequencing of the molecules with IGF activity have revealed that two closely related peptides of approximately 7.5 kDa mediate these biological activities. At the amino acid level, IGF-I and IGF-II share approximately 70% identity and are also approximately 50% identical to proinsulin. Because serum concentrations of IGF-I, but not IGF-II, are primarily regulated by GH, the term "somatomedin" does not adequately describe these peptides, and, consequently, these peptides are now referred to as IGF. Insulin-like growth factor-I functions as an anabolic agent, primarily in postnatal life, and IGF-II plays a comparable role in the fetus (97).

IGF-I and Lymphoid Tissues

Because the discovery of the IGF peptide family is more recent than that of the somatolactogens, the literature describing effects on lymphoid tissue is consequently less abundant. Nonetheless, growing evidence suggests that IGF-I affects a comparable array of immune events. A generalized effect of IGF-I on lymphoid organ size and cellularity was reported by Guler et al. (48), who found that subcutaneous infusions of IGF-I into hypophysectomized rats were more effective than GH treatment in restoring spleen and thymic size. Also, IGF-I has significant positive effects on Thy-1 antigen expression and thymocyte maturation in the atrophied thymus of diabetic rats (12). These results strongly suggest that many of the effects of GH on lymphoid tissue (55, 56) may be mediated by the paracrine synthesis of IGF-I.

IGF-I and Lymphocyte Function

Because cellular proliferation is one of the more accessible measurement parameters, a large body of data exists showing an effect of IGF-I on the unstimulated proliferation and mitogen-induced proliferation of a range of immune cell types. These in vitro results were heralded by the observation that the stimulating effect of serum on lymphocyte mitogenesis is increased in acromegalics and decreased in pituitary dwarfs (99). Insulin-like growth factor-I has anabolic effects, such as the induction of macromolecular synthesis, and enhancement of intermediary metabolism (105) and DNA synthesis (99) in mitogen-activated lymphocytes cultured in suboptimal serum concentrations. The latter results have since been confirmed in a variety of systems [e.g., purified human T cells (53, 103), human leukemic blasts (36, 86, 100, 101), and murine and human thymic lymphoma cells (44, 101)]. However, the findings were not confirmed, by Rao et al. (89) or by Verland and Gammeltoft (105); indeed, immunosuppressive effects of IGF-I have also been demonstrated (51). These divergent findings are difficult to reconcile, but at least some of the discrepancies can be explained by the culture method in which the inclusion of serum with its high content of IGF-I and IGF-binding proteins is a confounding factor. In some instances demonstrating enhancement of proliferation by IGF-I, the specificity of the signaling pathway has been demonstrated by the use of an antibody directed against the human type I IGF receptor, which abrogates the enhancement mediated by IGF-I (43, 53, 86, 100).

IGF-I Involvement in Hematopoiesis and Granulopoiesis

Some of the earliest evidence of a role of IGF-I in modulation of immune events came from studies demonstrating an effect of rela-

tively impure somatomedin preparations on hemopoietic cells. Generally, colony formation of erythroid cells from embryonic mouse liver and adult bone marrow was stimulated by physiological IGF-I concentrations (62). More recently, these findings were corroborated in a number of laboratories with recombinant IGF-I. Phillips et al. (87) treated neonatal rats with IGF-I in vivo and found a significant increase in bone marrow erythropoietic cell precursors. Kurtz et al. (63) found that IGF-I stimulated erythropoiesis in hypophysectomized rats directly and also indirectly through the stimulation of increased erythropoietin. Werther et al. (112) and others (64) confirmed these findings by demonstrating that these growth-promoting effects were seen in vitro in the absence of either serum or erythropoietin. Reliable evidence now suggests that the effects of GH on erythropoiesis (5), which include the promotion of primitive and mature erythroid precursors, appear to be mediated by monocyte-derived paracrine IGF-I. The GH-mediated enhancement of erythropoiesis was abrogated by an antibody directed to the IGF-I receptor (69), and granulopoiesis was enhanced in vitro by GH and IGF-I (70), suggesting that IGF-I may have generalized growth-promoting effects on a range of hemopoietic precursors. Enhancement by GH in the latter instance was dependent on the presence of bone marrow adherent cells, presumably as a source of paracrine IGF-I, and was abrogated by an antibody directed against the IGF-I receptor. A possible mechanism for the effects of IGF-I in differentiating myeloid cells was suggested by the recent demonstration that this growth factor prevented apoptosis in bone marrow lineages derived from IL-3 upon removal of this cytokine (91), suggesting that IGF-I maintains proliferative capacity in differentiating progenitors.

IGF-I and Phagocyte Effector Functions

Our group (28, 41) has recently described a novel effect of IGF-I on phagocyte effector functions; IGF-I and GH were equally as potent as IFN-γ in eliciting macrophage and granulocyte superoxide anion production. These results complete the characterization of a striking range of actions for these growth-promoting peptides on granulocytes and macrophages. Thus, GH and IGF-I seem to be comparable in their range of activities to the colony-stimulating factors (e.g., granulocyte-colony stimulating factor) and granulocyte-macrophage colony-stimulating factor, which can influence the differentiation and the effector functions of phagocytic cells. At least in the case of human PMN, GH or PRL priming does not appear to be mediated by induction of autocrine IGF-I production because an antibody to the IGF-I receptor abrogated priming mediated by IGF-I, but not GH or PRL (40).

Production of Somatolactogens and Somatomedins by Immune Cells

A central tenet of the neuroendocrine-immune axis is that bidirectional communication signals and a shared system of communication ligands and receptors exist between these systems. A complete review of the now voluminous data supporting the existence of this network is outside the scope of this article but has been presented elsewhere (13, 21, 58, 108, 199). However, some comments on the emerging concept of an autocrine-paracrine somatolactogen-somatomedin circuit in immune cells are pertinent. Prolactin production by cells of the immune system was first demonstrated in murine splenocytes treated with concanavalin A (75). Subsequently, a PRL-like protein was shown by morphological and biochemical approaches (59, 74) to be present in murine splenocytes. Recently, in cooperation with colleagues at The Ohio State University, we (96) found PRL mRNA and immunoreactive PRL in human peripheral blood lymphocytes, suggesting that this molecule functions as an autocrine mitogen in lymphocyte proliferation. An elegant series of studies by Clevenger et al. (19) confirmed this postulate. The presence of an immunoreactive GH-like molecule, similar to pituitary GH in molecular weight, antigenicity, and bioactivity in lymphocyte-conditioned supernatants, was first reported by Weigent et al. (107). Endogenous GH secretion by resting and mitogen-stimulated peripheral blood mononuclear cells can be augmented by exogenous GH (49), and an antisense oligonucleotide to GH mRNA abrogates lymphocyte proliferation (111). Paracrine regulation of lymphocyte GH production is strongly suggested by the

demonstration that lymphocytes produce an immunoreactive GH-releasing hormone (110) and express mRNA transcripts for somatostatin (42).

Localized synthesis of IGF-I by immune tissues was first suggested by the immunohistochemical demonstration by D'Ercole et al. (26) of IGF-I in the thymus of adult rats in which its concentration was decreased by 60% following hypophysectomy. Geffner et al. (43) also provided convincing data that the enhancement of T-lymphoblast cell lines by GH was actually mediated by local synthesis of IGF-I and could be abrogated by antibodies directed against either IGF-I or the IGF-I receptor. Merimee et al. (72) found evidence for a similar GH responsive IGF-I paracrine circuit in B cells, and Merchav et al. (69, 70) and Werther et al. (112) found that GH enhancement of erythropoiesis was mediated through local synthesis of IGF-I. Support for such an autocrine-paracrine circuit is also provided by the demonstration of Neely et al. (82) that some human leukemic T and B lymphoblasts produce insulin-like–binding proteins 2 and 4, suggesting that these cell types can regulate IGF-I concentrations in their microenvironment. Comparable studies on nontransformed immune cells have not been reported. Baxter et al. (3) have demonstrated GH responsive IGF-I bioactivity in murine splenocytes. Rom et al. (92) characterized a growth factor for fibroblasts from alveolar macrophages of humans suffering from asbestosis. This alveolar macrophage-derived growth factor had an apparent molecular mass of 26 kDa but displaced ^{125}I-labeled IGF-I from its receptor in a binding assay and stimulated IGF-I receptors to phosphorylate an artificial substrate containing tyrosine. In situations of lung pathology, this IGF-I bioactivity could promote potentially damaging fibroblast proliferation. Data from this and other groups (78, 90) suggested that IGF-I mRNA transcripts were particularly abundant in macrophages. The appearance of IGF-I mRNA transcripts in wound macrophages supports a role for this peptide in wound healing (97). We have examined a variety of immune tissues, using a sensitive ribonuclease protection assay, and found that all cell types produced detectable IGF-I mRNA transcripts but that these were particularly abundant in macrophages undergoing differentiation (Figure 4; Arkins and Kelley, unpublished results). A recent description of the in vivo and in vitro downregulatory effects of the pleiotropic inflammatory mediator, IL-1β, on Leydig cell IGF-I gene expression and steroidogenesis (68) also argues for the existence of a shared regulatory pathway involving cytokines and the autocrine-paracrine production of IGF-I.

CONCLUSIONS

The immune system can no longer be conceived of as being either self-regulated or insensitive to the host's internal and external environments. One important application from the study of immunophysiology has been the increased understanding of the effects of environmental stress on the immune system of farm animals (54, 58). The knowledge that glucocorticoids are potent immunosuppressive agents has had significant implications for approaches to animal management. Also evident, however, is that all stressors are not necessarily immunosuppressive and that neuroendocrine hormones can also have positive influences on immunocompetence. Indeed, we (21, 58) have previously suggested that somatolactogens may counteract the immunosuppressive effects of glucocorticoids in vivo, and, at least in the case of PRL, evidence is steadily accumulating to support this postulate (9).

A review of the literature indicates that the immunomodulatory effects of GH and PRL are more marked in hypopituitary or old animals than in young animals. Comparable in vivo studies have not yet been reported for IGF-I. Nonetheless, in situations in which animals are treated with antiserum to GH or subjected to pharmacological suppression of PRL, a number of clearly defined immunodeficiencies ensue. Unfortunately, very little is known about the molecular mechanisms by which GH, PRL, or IGF-I exert their immunomodulatory effects. A number of distinct scenarios are possible. In the case of the somatolactogens, immunomodulatory effects might be mediated indirectly by alteration of the synthesis or activities of macrophage-derived proteins, such as IL-1 or TNF-α or T- cell products such as IFN-γ. Alternatively, the somatolactogens may play a role in vivo as immunopermissive hor-

mones, countering the suppressive effects of glucocorticoids on immune tissues (21, 58). This hypothesis is certainly supported Bernton et al. (9), who demonstrated that GH and PRL protected mice in vivo from the immunosuppressive effects of corticosterone. A third possibility is that GH and PRL act on immune tissues through the induction of an intermediate, such as IGF-I, that has clear functional roles in cell cycle events (102) and in the differentiational and maturational phases of a variety of cell types (38, 85, 91). The induction of one or more intermediates would certainly agree with the pleiotropic range of activities now described for these hormones in immune cells.

Although the immunological effects of somatolactogens and somatomedins have not been explored extensively in domestic livestock, preliminary results suggest that recombinant bovine growth hormone enhances T-cell proliferation (14, 15), augments the number of circulating neutrophils (14, 17), increases the production of reactive oxygen intermediates (18, 50), and reduces the clinical symptoms of acute experimental *E. coli* mastitis in dairy cows (58). From the results of studies in laboratory animals, these immunomodulatory properties are likely best exploited in situations of environmental or metabolic stress. The challenge for future studies is to establish the molecular mechanisms by which these hor-

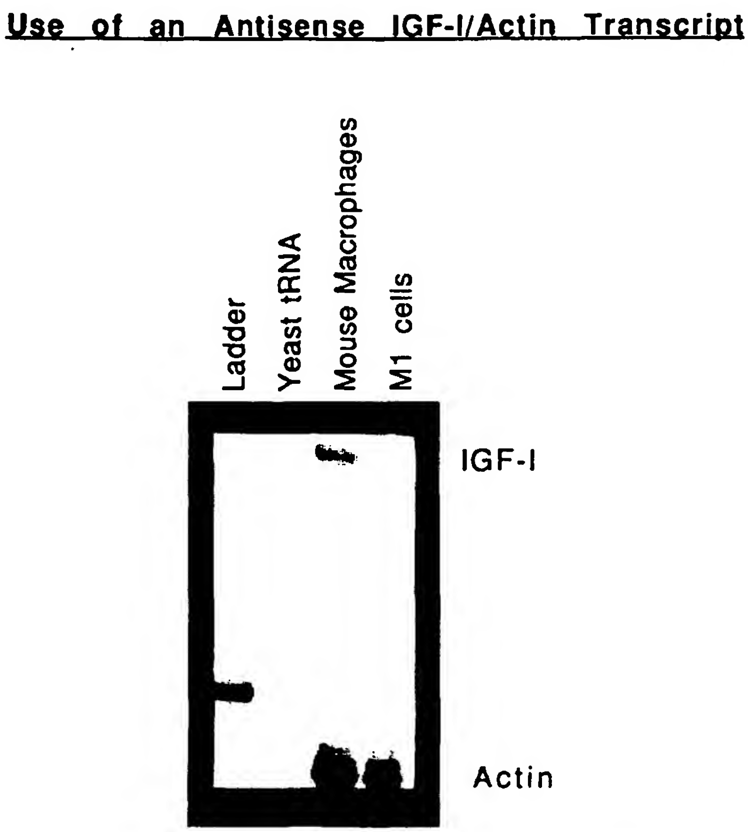

Figure 4. An antisense RNA transcript to IGF-I exon 4 protects a 182-nucleotide fragment in total RNA from murine macrophage but does not detect IGF-I transcripts in the murine premyeloid cell line (M1). Yeast tRNA was included as a negative control. Samples were simultaneously hybridized with a 115-nucleotide antisense RNA transcript to β-actin as a control for RNA integrity and loading accuracy. (From S. Arkins and K. W. Kelley, unpublished data.)

mones affect immune events and to determine how to exploit their immunomodulatory potential.

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